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Botulinum Toxin Type B: Experimental and Clinical Experience

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INTRODUCTION

Botulinum toxins BTXs (types A, B, C1, D, E, F, and G) are among the most potent toxins known. The toxins have been studied since the turn of the century, initially to gain an understanding of botulism, a form of food poisoning. Later, they were studied as something of a curiosity, because of the uniquely long-lasting and specific muscle paralysis induced by minute amounts of the toxins. Today, that "curiosity" is beginning to be exploited in the treatment of movement disorders such as blepharospasm and torticollis.

The type B toxin is possibly the first BTX ever discovered, having been identified as the causative agent in a 1895 outbreak of botulism (1). In this incident, which took place in Ellezelles, Belgium, three musicians died. The cause of death appeared to be a neuroparalytic toxin produced by an anaerobic, spore-forming bacterium. When these bacteria were cultured, the culture medium was found to cause botulism-like toxicity in a variety of experimental animals, by various routes of administration. It was later found that this toxicity was not prevented by protective antisera produced against toxin from bacteria isolated from a different incident of botulism, in Germany. Similarly, antisera protective against the Belgian toxin were not protective against the German toxin, although the bacteria that yielded the two toxins, and the toxicity they produced, were similar (2). Although both cultures were later lost, it is believed that the second type of *Clostridium botulinum* was a strain of what we today call type A (3). Thus began a long list of publications related to the bacteria and their toxins: there are far more publications related to BTXs in the following years than there have been documented cases of human botulism.

1.

STRUCTURE OF BOTULINUM TOXIN TYPE B

Toxin–Nontoxic Protein Complex

As isolated from the bacterial culture medium, type B toxin (like type A toxin) is found combined with nontoxin proteins. In the case of type B toxin, stable complexes of two different sizes are formed. These complexes have sedimentation coefficients of 16 and 12 S, and are called L (large) and M (medium), respectively. (The uncomplexed, pure toxin protein is sometimes called the S, or small, form.) The M complex contains nontoxin proteins that reportedly do not have hemagglutinin activity (4). The larger of the two types of complex (L form, about 450–500 kD) contains other protein(s), in addition to these nontoxin proteins, that do have hemagglutinin activity. These toxin–nontoxin protein complexes are not held together by covalent bonds and can reversibly dissociate, releasing the toxin protein, depending on the pH and ionic strength of the solution. Both pH greater than 8 and low ionic strength favor dissociation of the complex. There is some antigenic cross-reactivity and sequence homology between the hemagglutinins of the type A and B toxins (5). However, neutralization of type B hemagglutinin activity with a type A antitoxin prepared against a type A toxin–hemagglutinin complex does not neutralize the type B toxin (6). Formation of an association complex with the nontoxin proteins appears to stabilize the activity of BTXs, perhaps by helping to maintain a necessary secondary or tertiary structure (7). It is presumably for this reason that the only currently commercially
102 available BTX for clinical use, type A, is formulated in the form of a toxin–hemagglutinin-containing nontoxin protein complex, rather than as a formulation of the pure toxin.

Toxin

The type B toxin is synthesized by the bacteria as a single protein chain that has low activity until proteolytically cleaved. This cleavage—"nicking"—occurs endogenously by the action of bacterially produced protease(s) (8,9). Strains that activate most of the produced toxin are termed "proteolytic," such as the strains "Beans" and "Okra." Full activation of the toxin can also be achieved artificially by trypsinization: the bacterial nicking protease and trypsin cleave at or very near the same site. The amount of endogenous activation varies according to the *C. botulinum* type B strain and the fermentation conditions. Even under the best conditions, using the proteolytic strains, both nicked and unnicked toxin may be produced (10).

The nicked toxin has a molecular weight of about 150,000 kD and is composed of a heavy and light chain, held together by a disulfide bond and noncovalent bonding. Reduction of the disulfide bond causes a separation of the chains and loss of toxicity (11): neither chain by itself is toxic. The molecular weights of the two chains are about 100,000 and 50,000 kD, respectively (12). The nicking site is one-third of the length of the single chain, and the light chain is formed from the amino-terminal portion. The amino-terminal sequences of the unnicked type B are identical to the amino-terminus of the light chain of the nicked toxin (13). Whether this proteolytic cleavage termed "nicking" is sufficient to activate the toxin remains controversial (14).

Until very recently, only limited structural information about the type B toxin was available. Most of this information was derived by inference from antigenicity studies (although there was a limited amount of sequence data obtained from fragments of the toxin). The antigenicity data suggested significant differences between the various types

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of BTX in terms of their amino acid sequences. The antigenic differences are so significant and reproducible, in fact, that the botulinum bacteria are primarily classified by the antigenic specificity of the toxins they produce, with additional subdivision by group and by strain. Assignment to group is based on slight differences in culture characteristics (proteolytic ability, heat resistance of spores, optimal temperature for growth). Various type B bacterial strains within these groups have been described, primarily on the basis of the first source of culture. Some amino acid sequence differences between type B toxin produced by different strains of type B *C. botulinum* (15,16) have been reported, and even, in some cases (depending on the strain), antigenic differences (17). However, neutralizing type B antitoxin raised against one strain of type B bacterial toxin is protective against type B toxin produced from a variety of strains of type B bacteria (18).

The structure of the type B toxin is loosely related to that of tetanus toxin, also produced by bacteria of the genus *Clostridium*. The general size and subunit structure of the two classes of toxin are similar, and some aspects of mechanism of action also appear to be comparable. Antipeptide antibody binding studies indicate some limited sequence homology exists between various BTXs and tetanus toxin, particularly in specific regions of the purified (possibly partially unfolded) proteins (19). However, human and mouse polyclonal antibodies that neutralize tetanus toxicity do not cross-react with the BTXs. This implies that the homologous sequences in the primary structure of tetanus and botulinum toxins are generally short amino acid segments that are either not accessible or not immunogenic in the native conformation state of the molecules. The functional implications of the sequence homology are not yet understood but may be of great interest in understanding the mechanism of action of the various toxins.

Recently, two groups (20,21) have reported sequence information about the type B toxin, from two different type B strains. Comparable information was already available for the other types of BTX and for tetanus toxin. A comparison of the type B sequence with the sequence of these other toxins indicates that the heavy chain of type B toxin produced by the Danish strain has a sequence homology of 48% to the heavy chain of type A toxin and 35% homology to the equivalent portion of the tetanus toxin sequence. A hydrophilicity plot comparison of various strains of BTX types and of tetanus toxin suggests that for all of them, there is a conserved hydrophobic region in the heavy chain. This region may be important for translocation of the toxin across the extracellular membrane (see below). In contrast, the portion of the toxins that has previously been hypothesized to be most important to receptor binding, the heavy chain carboxyl-terminal end, is one of the regions of most divergent sequence. The sequence difference in this portion of the toxins could explain the specificity of receptor binding reported for the various types of clostridial toxins.

The light chain of the type B toxin produced by the Danish and Okra strains have 31–32% homology to the light chain of type A toxin as reported by these two groups (20,21). Surprisingly, the type B toxin was also found to have a 50–52% homology to the equivalent portion of the tetanus toxin sequence. The light chains of type A, C1, and D BTXs have a lower (roughly 30%) homology to the equivalent portion of the tetanus toxin. Analysis of the light chain sequences of the various types of BTX and of tetanus toxin also indicates a highly conserved region that contains a histidine-rich sequence. This sequence is typical of the active site of metalloproteinases, and it is tempting to assume that such activity could have some role in the intracellular action of the toxins.

PHARMACOLOGY AND TOXICOLOGY OF BOTULINUM TOXIN TYPE B

Mode of Action

Botulinum toxins, including type B toxin, probably act through a three-step process: extracellular binding onto the neuron, internalization, and intracellular poisoning (22). Binding appears to occur as a result of an interaction with specific acceptors on the surface of the presynaptic motor nerve terminal membrane (23). Using radiolabeled toxins and various in vitro/ex vivo models, distinct acceptor sites on the presynaptic terminal for some of the toxins have been found. Using rat cerebrocortical synaptosomes, sites specific for types A, B, E, and F have been reported: the sites are saturable, and toxins of one type bind weakly, if at all, to the acceptors of another type (24). In studies using mouse hemidiaphragms, type B toxin was not found to affect type A binding at all. (However, Black and Dolly reported that a large excess of type A toxin slightly reduced type B toxin binding to mouse hemidiaphragms in vitro [25]). Evaluating binding affinities with rat brain synaptosomes, Evans et al. found that there are two populations of acceptors for type B toxin: a smaller number of high-affinity sites (K_D = 0.3–0.5 nM; B_{max} about 30–60 fmol/mg protein), and a larger number of low-affinity sites (K_D = 16–21 nM; B_{max} > > 3000 fmol/mg protein) (26). In mouse hemidiaphragms, the total density of acceptors for type B toxin (627 ± 21% sites/μm^2) is approximately four times that of acceptors for type A toxin (152 ± 20% sites/μm^2) by electron microscope autoradiography (25).

Binding of the various BTXs to neurons is mediated by the carbonyl end of their heavy chain (27). Both the single-chain unnicked toxin and the heavy chain of type B toxin bind to rat brain synaptosomes, and the heavy chain is a potent inhibitor of the binding of the single chain, whereas the light chain is much less effective (28). Preincubation with the heavy chain of type B toxin antagonizes the in vitro paralysis of mouse hemidiaphragm induced by the active type B dichain (29).

Botulinum toxin binding to the presynaptic nerve membrane may involve both membrane determinants containing sialic acid (probably gangliosides) and one or more proteins (acceptor/receptor proteins) on the neuronal surface membrane (30). The acceptors for BTXs have not yet been isolated or characterized, and the evidence for this double receptor hypothesis is indirect, particularly for the role of gangliosides. Gangliosides, but not other membrane lipids, inactivate BTX in vitro and in vivo (31). Certain lectins with affinity for sialic acid–containing sugars reduce the binding of BTXs to brain membranes and reduce the neuromuscular blocking activity of the toxins (32). The specific gangliosides involved may differ between the various types of BTX. Type A toxin binds avidly to GQ_{1b} gangliosides, whereas type B toxin binds less efficiently to GQ_{1b} ganglioside (33), and more efficiently to GD_{1a} and GT_{1b} gangliosides than type A toxin (34). The results of these studies are highly dependent on the pH and ionic strength of the medium, however. Less is known about the protein component of BTX binding to neurons. It is presumed that there is such a component, and that it has functional significance, by analogy to other, better-characterized receptors. Also, toxin binding to synaptosomal membranes is affected by pretreatment of the membranes with either neuraminidase (attacking the sialic acid residues of the gangliosides) or proteases (35). This combination of neuronal-specific phospholipid and receptor protein requirements may help explain the very specific neuronal affinity of the botulinum toxins.

Binding of the toxin to its acceptor is neither sufficient nor, under experimental conditions, necessary to cause paralysis. Intracellular toxicity of the toxins can be

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observed using methods that bypass the binding step. Such methods include intracellular injection of the toxin into *Aplysia* ganglia (36), or using permeabilized cells (37). Similarly, there appears to be a time lag between in vitro binding of the toxin, when toxin antibody is protective, and subsequent paralysis (38).

A number of studies, including the histological studies cited above, have shown that the toxin is internalized after binding to the cell surface. For example, in vitro kinetic studies using mouse hemidiaphragm and antibodies to BTX revealed that after a while, toxin bound to the cell surface became inaccessible to the antibodies. The toxin is therefore presumably internalized (thus inaccessible) before the onset of paralysis (39). More direct evidence was provided by electron microscopy autoradiography, which showed that the toxin appeared to be internalized, in vacuole-like structures (25).

The internalization process is not completely understood, but, like binding, seems to require the presence of the heavy chain of the toxin. After binding, the toxin/receptor complex is taken into an endosome by an active, temperature-dependent process, and the toxin then somehow enters into the cytosol. The endosome has a lower pH than the extracellular milieu, and the lower pH appears to induce a conformational change in the toxin. As a result of the conformational change, hydrophobic domains are exposed that interact more extensively with lipids, as demonstrated using a liposome model system (40). It is hypothesized that the highly conserved hydrophobic region of the heavy chain inserts into the neuronal membrane and forms a channel, allowing some or all of the toxin molecule into the cell. At least in the case of the type B toxin, using a planar lipid bilayer model, it appears that at acidic pH, the heavy chain is capable of forming channels (41).

Inhibition of neurotransmitter secretion is probably caused by a site on the light chain (42). The mechanism by which intracellular poisoning is achieved is unknown for BTX-B, but it is generally believed to involve enzymatic activity and not mass action or receptor occupancy effects. This hypothesis is attractive for two reasons. First, since the paralysis induced by minuscule amounts of the toxin lasts for weeks or months, it seems likely that the toxin must act on an intracellular component by some means that is only slowly reversed. Such changes would most likely be catalyzed by an enzyme. Second, an enzymatic mechanism seems likely by extrapolation from the more clearly understood mechanism of action of other bacterial toxins, such as ricin and diphtheria toxin. These toxins have dichain structures and apparent structure/function activities generally similar to those of BTXs. The heavy chains of ricin and diphtheria toxin are also involved in binding and internalization, and the light chain is the portion with intracellular activity. In these cases, the activity has been demonstrated to be enzymatic (43). Botulinum toxins types C1 and D have been shown to ADP-ribosylate a membrane protein in mouse synaptosomes (44), but until recently there was no convincing evidence that enzymatic activity was associated with the other BTX serotypes, including type B. As noted above, there is some recently discovered homology between a segment of the amino acid sequence in the central core of the light chain of the various BTXs and zinc-dependent metalloproteinases. This sequence contains a histidine-rich motif that for the metalloproteases represents a zinc-binding site and part of the active site of the protease. Initial experiments using type A toxin mutants in which the histidine site has been affected have not shown any effect of the mutations on the effectiveness of BTX in *Aplysia* neurons (45). These results therefore do not support the hypothesis that this putative metalloprotease active site is important to BTX intracellular activity. At the same time, chelating agents such as 1,10-phenanthroline, which are capable of stripping zinc, iron, and calcium from proteins, are capable of inactivating BTXs including type B toxin (46).

All of the BTX serotypes prevent both spontaneous and evoked quantal release of acetylcholine from the presynaptic neuromuscular junction. However, the intracellular mechanism by which this effect is achieved appears to be different. A series of studies has been performed that emphasizes these differences, in synaptosomal preparations, in phrenic nerve–hemidiaphragms, and also in a double-poisoning experiment using types A and B toxins on a triangularis sterni nerve-muscle preparation (47). These studies all suggest differences between the toxins in terms of the response to pharmacologic manipulation of calcium ion movement, or to microtubule-dissociating drugs.

The most frequently studied difference between the toxins is reversibility of acetylcholine release inhibition by the aminopyridines. These agents increase impulse-evoked Ca^{2+} influx by blocking presynaptic potassium channels. As a result, neurotransmitter release can take place. Aminopyridines reverse the poisoning induced by types A and E (48), but the effect is greatest with type A toxin. The poisoning induced by B, D, and F is not reversible by aminopyridines. An increase in extracellular calcium or use of a Ca^{2+} ionophore, A23187 (49), or guanidine more effectively reverses the effects of type A than of type B, C, or E toxins (50). When synaptosomes were preincubated with microtubule-dissociating drugs, there was a reduction of type B (but not of type A) toxin inhibition of neurotransmitter release. This effect did not appear to be related to prevention of binding or internalization of the toxin (49). The most probable explanation for these differences is that the toxins act at different steps in the process that results in neurotransmitter release.

Comparative Muscle Paralytic Efficacy

Relatively few studies have been reported evaluating the in vivo muscle paralytic potency of BTX types other than type A. Usually, these studies have been performed using rats, a species apparently particularly resistant to type B toxin (see below), or rat tissues. In a study by Burgen et al. using a rat in vitro phrenic nerve–diaphragm preparation, when the toxin was added to the perfusate of the in vitro system, it took amounts of type B toxin ". . . about 500 times greater than the amount of type A toxin required to produce a similar rate of paralysis. When, however, the phrenic nerve–diaphragm preparation was obtained from young guinea-pigs (150–200 g.) typical neuromuscular block followed the addition of 2000 units/ml. of either type A or type B toxin. There were no marked differences in the latent period or rate of paralysis between types A and B toxins on this preparation. The guinea pig is known to be susceptible to type B toxin. . ." (51).

Another study, by Sellin et al. (52), found that it required 1200 mouse LD_{50} of type B toxin to prevent measurable evoked potentials in a rat ex vivo nerve-muscle preparation. The difference between these two studies in terms of the effective dose for type B toxin–induced paralysis may be that Sellin et al. administered the toxin subcutaneously, above the tibialis anterior muscle in vivo, then later removed the extensor digitorum longus muscle to measure in vitro electrophysiological effects, while Burgen et al. added the toxin to the perfusate of their in vitro preparation.

In rats, no toxin studied has been found to be as potent as type A. Type E (53) and F (54) toxins have also been studied with the same model, and it was found that the dose and duration of the paralysis induced in rats was less than with type A. Unfortunately, given the species-specificity of the various toxin types, rodent studies must be considered inconclusive with respect to predicting the relative clinical potency of the various types of BTX.

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For this reason, we have undertaken to study the paralytic efficacy of BTX-B in normal nonhuman primates. In these studies, the toxin was injected into three muscles: the trapezius, the abductor pollicis brevis (APB) muscle of the thumb, and the extensor digitorum brevis (EDB) muscle of the foot. Doses injected into muscles ranged from 5 to 80 U/muscle (1 U is the dose equivalent to a mouse intraperitoneal LD_{50} dose). Efficacy in these studies was measured electrophysiologically, by a decrease in the peak amplitude of the evoked compound muscle action potential. Botulinum toxin type B was found to paralyze injected muscles effectively. At 2 weeks after the initial injection, there was a reduction of maximal muscle electromyographic (EMG) amplitude of 80% or more in the APB and EDB muscles injected at all of the doses tested, in all subjects. A similar reduction of maximal trapezius muscle EMG amplitude was observed after 2 weeks in 10 of 11 animals injected with doses greater than or equal to 80 U/muscle. Muscles paralyzed by BTX-B recovered over time. All of the muscles injected with doses in the estimated therapeutic range had evoked compound muscle action potentials that could be measured by approximately 3 months after the last injection. This result is in contrast to results obtained in the same animals 2 weeks after the initial injection, when several muscles had no measurable evoked compound muscle action potentials. On the basis of visual estimates of the mass of the three muscles tested (the actual mass of the injected muscles was not determined), it appeared that for any given dose, the smaller the muscle mass (abductor pollicis brevis < extensor digitorum brevis < trapezius), the longer the duration of paralysis. Evoked compound muscle action potentials in the injected APB tended to be lower in each animal at each injected dose than in the trapezius, although the dose of BTX-B injected into the APB was one-fourth of the trapezius dose. Similarly, whereas at the end of the study the evoked compound muscle action potentials in the APB and trapezius muscles in the lowest-efficacy dose group were no longer statistically significantly different from baseline values, the evoked compound muscle action potentials of both muscles in the highest-efficacy dose group were significantly less than at baseline.

Reinjection of recovering muscles with a second dose of BTX-B resulted in a decrease in the evoked compound muscle action potentials measured 2 weeks after administration of the second dose. The response of the muscle injected with the second dose of the drug did not indicate a cumulative effect of the doses: reinjected muscles were neither more completely paralyzed nor longer paralyzed than muscles injected with a single dose of the drug.

For type A toxin, histochemical studies have been made, evaluating the changes that occur after injection of the toxin. Duchen (55) performed a series of such studies, injecting the type A toxin into the calf muscles of mice. Typically, the muscles atrophied, as expected from a functional denervation. Also observed was a sprouting of nerve fibers and a formation of new end plates, which occurred more rapidly in the soleus (a slow-twitch red muscle) than in the gastrocnemius (a fast-twitch white muscle). The sprouting was observed to take place preterminally as well as terminally, and collateral sprouting was also observed. Over time, as the muscle recovered function, the number of these collateral sprouts decreased. Similar sprouting after type A toxin injection has been reported in frogs (56), primates (57), and humans (58).

The mechanism by which the type B toxin muscle paralysis is reversed over time is unknown, but it may be assumed to be similar to recovery after type A–induced paralysis. This conclusion is based on results of a study submitted for publication, cited in a recent review article (59). The review article reports that this study was performed in albino rabbits, evaluating histochemical changes (acetylcholinesterase stain, muscle fiber size,

and ATPase staining) after injection of either BTX-A or a "crude preparation" of type B toxin." The denervation indicated by histochemical staining and fiber size analysis appeared transient and lasted for about 3 months for both type A and B toxins."

Comparative Toxicity Data

Type B toxin is known to be toxic for humans, as noted in the introduction, when ingested in poorly processed foods. In the United States, most reported cases of botulism have been related to either type A or type B toxin: together they accounted for 91% of the cases of known toxin-type botulism reported to the Centers for Disease Control from 1970 to 1979. Type A toxin is responsible for twice as many cases of food botulism as type B (60). Of the two types of human botulism, type A is considered to be the more severe (61). Human oral toxic doses are difficult to estimate from these oral food poisoning reports, however, and the clinically toxic dose, even by the oral route, is unknown.

Typically, it takes 1 to 2 days for the symptoms of botulism to develop. The highest cranial nerves are affected first, causing medial rectus paresis, ptosis, and sluggish pupillary response to light. They are followed by the lower cranial nerves, then the peripheral motor neurons, finally and often fatally including those that innervate the respiratory muscles. Effects on peripheral muscles are not observed in the absence of ophthalmic changes: in one study of an unusually large outbreak of type B botulism (53 people were hospitalized), all of the patients who later experienced respiratory difficulty or peripheral muscle weakness first demonstrated cranial nerve impairment (62). In some cases, patients have signs of autonomic nervous system dysfunction: constipation, distention of the urinary bladder, and decreased salivation and tearing (63). Blood counts, urinalysis, and clinical chemistry values are normal in botulism, unless there are secondary complications (the most frequent of which is pneumonia associated with respiratory muscle paralysis) (64).

A definitive diagnosis of botulism can be made by electromyography (EMG). In muscles weakened by botulism, the amplitude of the compound muscle action potential is reduced in response to a supramaximal stimulation of the nerve (65). In partially paralyzed muscles, the response to a double stimulus shows an incremental response (66). However, in severely paralyzed muscles, the neuromuscular blockade may be so complete that repetitive stimulation may not be effective (67). Other EMG measurements appear to remain normal in botulism patients: in a report of an extensive EMG study made in a single type B botulism patient, the latency of facial nerve and upper limb motor and sensory conduction velocities remained normal (68).

Within- and between-species toxicity data from various sources for the type B toxin are difficult to compare, for several reasons:

1. Different end points (minimum lethal dose versus LD_{50}); reference species used (in some cases, the data are expressed as mouse lethal units, in other cases, as guinea pig lethal units).
2. Different normalizing units: units per milligram total nitrogen, per absorbance at different wavelengths, per milligram protein measured using different assays. (Interconversions between these units of measure are approximations at best.)
3. Probable differences in the purity of the toxin and/or percentage nicked versus unnicked toxin in the test material.
4. Differences in route of administration: intraperitoneal, subcutaneous, intravenous, oral, even inhaled.

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However, as reported even in the earliest studies by van Ermengem, there appears to be species-specificity in terms of the relative sensitivity to type B toxin, with guinea pigs more sensitive to type B toxin than rats or rabbits (69).

Route of administration also appears to make a difference, at least in terms of the amount of the drug required for toxicity by various routes of administration. This should not be surprising, as pointed out in a paper by Lamanna (70):

> Poisoning of an animal by any mode of exposure to a toxin, including inhalation, will depend on the outcome of a sequence of four events: first, the capacity of the toxin to escape destruction inherent in the techniques of application; second, the capacity of the toxin to get to and to remain at the site of deposition where effective systemic absorption can take place; third, the capacity of the toxin after absorption to be transported to the primary site of poisoning; and fourth, the capacity of the toxin to resist specific or nonspecific forces of detoxification on route to the sites of action.

In this paper, for type B toxin, the rapidity of death (presumably inversely proportional to the amount of toxin required for overt toxicity) in rabbits appeared to differ significantly depending on the route of administration. The data, taken from an older article (71), are in Table 1. Note that time to death after injection of the toxin by the intraperitoneal route is significantly longer than after intramuscular injection. Intraperitoneal injection is the route typically used with mice to standardize the relative potency of various preparations of BTXs. Despite differences in the route of administration, the symptoms of poisoning were not apparently different. This is also consistent with toxicity data available for other BTXs.

A great difference in the relative toxicity of type A and type B toxins was also seen in the rat, in the study by Burgen et al. described above, where the LD_{50} (intraperitoneal injection) in 200 g rats of the two toxins were found to be approximately as follows: rat LD_{50}, type A toxin = 25 mouse LD_{50}; rat LD_{50}, type B toxin = 10,000 mouse LD_{50}. Sellin et al., in the comparable study, found that doses of type B toxin of 5000 mouse LD_{50}

Table 1 Order of Rapidity of Death of Rabbits by Various Routes of Injections of Botulinum Toxin Type B

	Time of death		
Route	days	hr	min
Intravenous		2	25
Intra-arterial		2	45
Intramuscular		4	30
Intracerebral		4	35
Intrapulmonary		5	0
Occipital		6	10
Subcutaneous		7	45
Eye anterior chamber		8	15
Intraperitoneal		9	5
Intrasciatic		10	53
Intragastric		33	45
Intrarectal	4	9	0

Source: From Legroux, Levaditi and Jéramec, 1945, as cited in Ref. 70.

caused debilitation or death (in contrast, only 20 mouse LD_{50} units of type A toxin caused similar toxicity). In the Lamanna paper, data were also given comparing the sensitivities of guinea pigs to different types BTX administered by various routes, as listed in Table 2. The doses in this table are expressed in mouse intraperitoneal units, in which one unit is the mouse LD_{50} dose by that route of administration. Whereas for BTX-A and BTX-B toxicity by the various routes is of the same order of magnitude, the type E toxin appeared to be much less toxic. Although no information about the bacterial source strains, level of purification, or pretreatment was provided, the difference is presumably due—at least in part—to the amount of nicking in the tested toxins. Type A toxin is always nicked by the organisms, type B may be (depending on whether the source strain was proteolytic or not), and type E toxin is not nicked endogenously.

In another report, using type B toxin crudely purified from a nonproteolytic strain (the toxin was therefore presumably mostly in an unnicked form), another comparison of route of administration versus test species was made (72). The results are summarized in Table 3. The data from this study suggest that the relative oral potency of this toxin preparation may have been much greater than for the toxin preparation cited by Lamanna. This may reflect the amount of nicking of the toxin (by the oral route, the toxin may have become activated in the gastrointestinal tract, while less activation occurred after parenteral administration).

No primate toxicity data for type B toxin were found in the literature, other than a single-animal, single-dose report. In this study, it was reported that for rhesus monkeys, oral administration of 100 guinea pig minimum lethal dose (least amount that will kill a 350 g guinea pig in 96 hours after subcutaneous injection) was lethal within 24 hours.

As part of our studies evaluating the effects of BTX-B in normal nonhuman primates, we also evaluated the potential toxicity of doses 10 to 20 times those that effectively paralyzed muscles. In these studies, the toxin was injected intramuscularly, in doses divided over five different muscles: the trapezius, abductor pollicis brevis, extensor digitorum brevis, gluteus maximus, and biceps femoris. Doses in this phase of the study ranged from total body doses of 120 to 480 U/kg total body weight. Toxicity was evaluated by clinical observations, clinical chemistries, ophthalmoscopic evaluations, electrocardiograms, and electrophysiological measurements: changes in peak evoked compound muscle action potential of muscles not injected with the drug (expected to be reduced if system weakening was caused by the drug), nerve conduction velocities of peripheral motor and sensory nerves (expected to change as a result of distal myelinopathies and axonopathies), and somatosensory evoked potentials to evaluate potential dysfunction

Table 2 Toxicity of Botulinum Toxins for the Guinea Pig by Various Routes

	Mouse intraperitoneal (ip) units		
Type	ip	oral	respiratory
A	5.2	840	141
B	4.8	413	350
E	78.0	456,000	778

Source: From M.A. Cardella and J.V. Jemski, personal communication, as cited in Ref. 70.

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Table 3 Relative Susceptibility of Mice, Guinea Pigs, and Rabbits to Type B Toxin Administered Subcutaneously, or Orally, as Multiples of Number of Guinea Pig Minimum Lethal Subcutaneous Doses

	Route of administration	
Species	Subcutaneous	Oral
Mice	0.2	2
Guinea pigs	1	2
Rabbits	20	100

Source: Based on data from Ref. 72.

in the central nervous system. No signs of toxicity were observed, even after injection of a total of 480 U/kg, either when administered in a single dosing or when administered as two separate doses of 240 U/kg given 11 weeks apart. Specifically, there were no signs of autonomic nervous system deficits, ophthalmic changes, difficulty in swallowing, or inability to maintain normal posture, even at the highest dose tested. Electrocardiographic changes initially observed in a preliminary segment of the study were found to be the result of the ketamine and pentobarbital anesthesia regimen, rather than related to the toxin itself. The EMG studies also did not indicate any central or peripheral neuropathy or systemic muscle weakness, even at the highest doses tested. In one of the test groups, a dose of 240 U/kg was reinjected into the same animals less than 3 months after an initial dose of 240 U/kg. Again, in these animals, no signs of toxicity were observed, according to the measures listed above.

CLINICAL USES OF BOTULINUM TOXIN TYPE B

The relative clinical value of BTX-A and BTX-B in the treatment of cervical dystonia, or other movement disorders, is currently unknown. The structural and pharmacologic differences between the types of toxin, summarized above, suggest that they might not be therapeutically equivalent. Such differences, if real, are likely to be demonstrated only after injection into dystonic muscles. However, even assuming no specific therapeutic benefit of one toxin type over the other, type B toxin could still be useful for this indication. Because the two toxins are antigenically different (antibodies to type A do not block the effects of type B toxin, and vice versa, in animals or in vitro models), the two toxins could be used together, or in rotation, thus reducing antigenic presentation of either toxin. This, in turn, could delay, reduce, or prevent the development of resistance to toxin therapy. In those patients who have already developed resistance to botulinum toxin type A, the type B toxin could provide the best treatment available.

While our studies in nonhuman primates suggest that BTX-B may be effective and have an excellent therapeutic index, the actual effectiveness of this drug can be judged only after use in dystonia patients. The extrapolation of these results from normal nonhuman primates to dystonic humans assumes no major difference in terms of sensitivity to this specific serotype of the toxin, and no unique difference in muscle responsiveness associated with dystonia as opposed to normal muscles.

SUMMARY

The type B botulinum toxin has many biochemical and biological similarities to other serotypes of BTX. At the same time, there are some indications that the type B toxin may have some unique properties that could have beneficial clinical implications. Most obvious of these is that its amino acid sequence is sufficiently different from those of other serotypes that type A–resistant patients may respond to type B. The mechanistic differences in the mode of action of the various types of BTX have not yet been intensively studied under conditions that will allow prediction of clinical benefit (if any) to dystonia patients. Most of these studies to date have been performed in normal rodents or using in vitro/ex vivo models, which, because of interspecies differences in sensitivity to various types of the toxin, may not be directly applicable. Preliminary data from our laboratory, however, suggests that type B toxin effectively paralyzes nonhuman primate muscle after intramuscular injection and is possibly less likely to cause systemic toxicity than type A toxin.

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